

It will be appreciated that the term marker gene is intended to include genes involved in specific biosynthetic pathways and/or genes involved in environmental tolerance.

Preferably, the marker gene is selected from the group consisting of *nptII*, *Ble*, *dhfr*,  
5 *cat*, *aphIV*, *SPT*, *aacC3*, *aacC4*, *bar*, *EPSP*, *bxn*, *psbA*, *tfdA*, *DHPS*, *AK*, *sul*, *crsI-1* and *tdc*.

Preferably, the method is capable of deleting in the region of up to 10 kb between each of the two attP regions and more preferably in the region of 7kb.

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Preferably, in the instance of removing more than one marker gene and/or vector sequence and/or other foreign ancillary nucleic acid each undesirable part of the transgene to be removed is flanked by att P regions. Thus it will be appreciated that the method of the invention can simultaneously be used to remove more than one  
15 undesired part of the genome at the same time.

Preferably, the attP region comprises 3052bp located between position 27492 and 27844 of bacteriophage  $\lambda$ .

20 Preferably the attP region comprises the nucleic acid sequence as set forth in SEQ ID NO:1, or fragment thereof with the same functional equivalent, or nucleic acids which hybridise under stringent conditions to the DNA of SEQ ID NO:1 and function as an attP region, or nucleic acids which differ from the DNA of SEQ ID NO:1 due to the degeneracy of the genetic code and which function as an attP region.

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The method of the invention provides a novel strategy to remove undesirable and/or other parts of a transgene after its integration into a plant genome. The method of the invention exploits the hitherto unrecognised potential of the high recombination efficiency of the attachment P region (attP) of bacteriophage  $\lambda$ , producing deletion  
30 events after intrachromosomal recombination between two attP regions. The attP system has been demonstrated to delete a 5.9kb region from a recombinant vector

CLAIMS

1. A method of removing a part of a transgene after its integration into a genome comprising flanking said part of the transgene on each side thereof with an attachment P region (attP) of bacteriophage  $\lambda$  and inducing intrachromosomal homologous recombination between flanking attP regions whereby said part of the transgene sandwiched therebetween is removed.  
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2. A method as claimed in Claim 1 characterised in that said transgene comprises a marker gene and/or vector sequence and/or other foreign ancillary nucleic acid.  
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3. A method as claimed in Claim 1 or Claim 2 characterised in that the marker gene confers resistance to antibiotics and/or herbicide resistance.  
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4. A method as claimed in any one of the preceding claims characterised in that the marker gene is involved in specific biosynthetic pathways and/or involved in environmental tolerance.  
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5. A method as claimed in any one of the preceding claims characterised in that the marker gene is selected from the group consisting of *nptII*, *Ble*, *dhfr*, *cat*, *aphIV*, *SPT*, *aaaC3*, *aaaC4*, *bar*, *EPSP*, *bxn*, *psbA*, *tfdA*, *DHPS*, *AK*, *sul*, *crs1-1* and *tdc*.  
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6. A method as claimed in any one of the preceding claims characterised in that more than one marker gene and/or vector sequence and/or foreign nucleic acid part is removed from the transgene and each such part is to be removed is flanked by an attP region.  
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7. A method as claimed in any one of the preceding claims characterised in that the attP region comprises 352 basepairs, or functionally equivalent fragment thereof, located between positions 27492 and 27844 of bacteriophage  $\lambda$ .

8. A method as claimed in any one of the preceding claims characterised in that the attP region comprises a nucleic acid sequence as set forth in SEQ ID NO:1 or functionally equivalent fragment thereof, or nucleic acids which hybridise under stringent conditions to the DNA of SEQ ID NO:1 and function as an attP region, or 5 nucleic acids which differ from the DNA of SEQ ID NO:1 due to the degeneracy of the genetic code and which function as an attP region.

9. A method as claimed in any one of the preceding claims characterised in that the attP regions are in a cassette.

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10. A method as claimed in Claim 9 characterised in that the cassette further includes a transformation booster sequence or fragment thereof for enhancing homologous and illegitimate recombination.

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11. A method as claimed in Claim 9 or Claim 10 characterised in that the cassette includes an effector gene such as oryzacyctastin-I or functional equivalent thereof.

12. A method as claimed in any one of the preceding claims characterised in that the genome is a plant genome.

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13. A plant or plant cell or plant tissue whenever produced by the method of any one of Claims 1 to 12.

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14. A method which comprises performing the method of Claim 12 to produce a plant or providing a plant or plant cell or plant tissue of Claim 13 and, in either case growing the plant and/or harvesting products therefrom.

15. A plant or plant cell or plant tissue comprising recombinant attP regions.

16. An attP recombination cassette comprising a marker gene and/or vector sequence and/or foreign ancillary nucleic acid flanked on either side by an attP region.
- 5 17. Use of an attP recombination cassette of Claim 16 for removing a part integrated into a plant genome.
18. A kit for removing a part of a transgene after its integration into a plant genome comprising an attP recombination cassette as claimed in Claim 16.
- 10 19. A plant or plant cell or plant tissue comprising a recombinant transgene integrated into its genome characterised in that the transgene is associated with a bacteriophage  $\lambda$  attP region on respective sides thereof.
- 15 20. A plant or plant cell or plant tissue as claimed in Claim 19 characterised in that it includes one such bacteriophage  $\lambda$  attP region and one effector transgene integrated into its genome.
21. A plant or plant cell or plant tissue as claimed in Claim 20 characterised in
- 20 that the bacteriophage  $\lambda$  attP regions and one transgene are not associated with a marker gene and/or vector sequence and/or other foreign ancillary nucleic acid.
22. A plant or plant cell or plant tissue as claimed in any one of Claims 19 to 21 characterised in that the transgene is further associated with a transformation booster sequence or fragment thereof which is capable of enhancing homologous and
- 25 illegitimate recombination.